Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Zilpaterol in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in laboratory quality distilled or deionized water to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety

Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

General Limited Warranty

Intended Use

The Beacon Zilpaterol Plate Kit is an immunoassay for the detection of Zilpaterol in EDTA whole blood samples. This product is intended for research use only.

Principles

Zilpaterol HRP Enzyme Conjugate is pipetted into the test wells followed by the Calibrators and the Sample Extract(s). A soluble polyclonal Zilpaterol antibody solution is then added to the test wells to initiate the reaction. During an incubation, Zilpaterol and Zilpaterol HRP Enzyme Conjugate compete for binding to the soluble Zilpaterol antibody which is in turn immobilized on the test wells. Following the incubation, the wells are washed to remove any unbound Zilpaterol and Zilpaterol HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Zilpaterol concentration of the sample is derived.

Reagents and Materials Provided

1 Unit	Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
4 X 2 mL	Vials of Zilpaterol Calibrators (0, 1, 7, and 40 ppb).
	Note: The calibrators actually contain 1/10th of the stated value to account for the 1:10 dilution during sample preparation. No further correction is required to obtain the concentration of Zilpaterol in the sample.
1 X 8 mL	Bottle of Zilpaterol HRP Enzyme Conjugate.
1 X 8 mL	Bottle of Zilpaterol Antibody.
1 X 50 mL	Bottle of 10X Wash Concentrate (dilute prior to use).
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Zilpaterol Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Zilpaterol Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Sample Preparation

EDTA Whole Blood: (Dilution Factor: 1)
1. Measure 2 mL of sample into a clean glass vial.
2. Measure 2 mL of acetonitrile and add to the vial.
3. Vortex for 30 seconds. Let sit for 10 minutes before proceeding.
4. Vortex for 1 minute. Let sit until a clean top layer appears.
5. Transfer 1 mL of the top layer to a microcentrifuge tube.
6. Centrifuge for 1 minute at 12,000 x g.
7. Dilute the supernatant 1:5 with laboratory quality distilled or deionized water.
8. Vortex to mix and use in the assay.

1X Wash Solution Preparation

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50 μL of Enzyme Conjugate** into each well.
4. Dispense **50 μL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 μL of Antibody** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **60 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution. Alternatively, the last wash can be done using laboratory quality distilled or deionized water to reduce interference associated with wash solution bubbles.
8. Dispense **100 μL of Substrate** into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
10. Dispense **100 μL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.